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Population-Based Longitudinal Study of Hepatitis B "e" Antigen Negative persons with Chronic Hepatitis B: Level of HBV DNA and Liver Disease

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Abstract

In a population-based cohort of Alaska Native persons with chronic HBV infection who were HBeAg-negative, we aimed to determine the prevalence of immune active HBV infection over time and to determine the relationship between demographic and viral factors on severity of disease on liver biopsy. We examined 777 patients infected with five HBV genotypes (A2, B6, C2, D2/3, F1). Alanine aminotransferase (ALT) measurements were assessed every 6 months during an 8-year period. HBV DNA levels were performed at baseline in 2001 and whenever ALT levels exceeded the upper limit of normal (ULN). Immune active chronic HBV infection was defined as an ALT >30 U/L men, >20 U/ml women and HBV DNA >2,000 IU/ml during one or more times from 2001 and 2008. Liver biopsies were scored using the modified hepatic activity index score (HAI) of Knodell and the Ishak fibrosis score. A total of 201 (26%) patients met the criteria for immune active HBV. HBV genotype D was less likely to be associated with immune active hepatitis than the other 4 genotypes. Of the 46 patients with liver biopsy results, none of the 15 patients with ALT always below twice the ULN and only 2/19 of those with HBV DNA between 2,000 and 20,000 IU/ml had moderate or severe hepatitis, or moderate or severe fibrosis. In contrast, 18(58%) of 31 with ALT ≥ twice ULN, and 16 (62%) of 26 with one or more HBV DNA levels > 20,000 IU/ml had moderate to severe fibrosis scores ($p<0.001$).

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Conclusion—Of participants, 26% met criteria for immune active HBV. An HBV DNA level of > 20,000 IU/ml was strongly correlated with persons meeting the current guidelines for antiviral therapy.

Keywords

HBV Genotype; Liver Biopsy; HBsAg levels

Chronic infection due to hepatitis B virus (HBV) (1) is a major cause of cirrhosis and hepatocellular carcinoma (HCC) worldwide. (2) Persons chronically infected with HBV experience four clinical phases of liver disease.(3) Persons that are hepatitis B “e” antigen (HBeAg) positive and have detectable viral load can be divided into two groups: those in the “immune tolerant phase” who have normal alanine aminotransferase (ALT) levels), and those in the “immune active phase” who have elevated ALT levels and active liver disease on biopsy.(4) Eventually, most persons will lose HBeAg and develop antibody to HBeAg (anti-HBe). The majority of these patients will enter the inactive carrier phase and have normal ALT levels, absent or low levels of HBV DNA and quiescent liver disease. Nevertheless, it is estimated that 20% to 40% either continue to have active liver inflammation and progressive fibrosis or revert from the inactive phase back to the immune active phase. However, the proportion of patients over time who will lose HBeAg and have active liver disease is not known, as there are few population-based prospective cohort studies that examine this question. Furthermore, factors that predict active vs. inactive HBV liver disease such as HBV genotype, levels of HBV DNA over time associated with disease, age and sex have not been well established.

For over 20 years, the Alaska Native Tribal Health Consortium Liver Disease and Hepatitis program has been conducting a large population-based cohort study of chronic HBV infection in Alaska. In the 1970s, Alaska Native people were found to have the highest rates of HBV infection and HCC of any U.S. born population.(5) Chronic HBV infection occurred in Alaska Native persons primarily through acquisition of HBV either at birth or in early childhood, as is seen in much of the developing world.(6) In the 1980s, 52,000 Alaska Native persons (approximately 75% of the overall population and >90% in the endemic geographic areas) were screened for HBV seromarkers, and approximately 40,000 persons negative for HBV seromarkers received Hepatitis B vaccine. Universal newborn hepatitis B immunization was also introduced at that time, and transmission of HBV in the population has almost ceased.(6) This public health program identified 1,536 persons with chronic HBV. In 2001, we began testing these patients for HBV DNA and continued to test for other HBV seromarkers and aminotransferase levels every 6 months. Herein we describe the prevalence of immune active liver disease in a cohort of 777 patients who were HBeAg-negative in 2001 and were followed through 2008. We examined the demographic and laboratory risk factors associated with the immune active phase, as well as the factors associated with the presence of moderate to severe liver disease. We also examined the levels of hepatitis B surface antigen (HBsAg) in persons who met the criteria for immune active hepatitis B to look for any correlations between level HBsAg and severity of liver inflammation and fibrosis at the time of biopsy.

Materials and Methods

Participants

Of the 1,536 Alaska Native persons chronically infected with HBV, defined as HBsAg-positive on two or more occasions at least 6 months apart, 1,271 persons consented to participate in this study. All Alaska Native persons, both consented and not consented, who are chronically infected with HBV have been contacted twice each year since 1982 and reminded to go to their village clinic or local hospital for a routine blood draw and follow-up testing. Seventy percent of Alaska Native persons with chronic HBV infection live in remote communities, most not connected by the road system. Blood specimens are centrifuged in the village and/or regional clinics and sent to the central laboratory at the Alaska Native Medical Center for testing. The results are reviewed weekly by a staff hepatologist and project nurse, who make decisions regarding further management, including laboratory tests that can be drawn in the village, and need for referral for radiographic studies and/or liver biopsy.

This study was approved by the Alaska Area Institutional Review Board and the Centers for Disease Control and Prevention Institutional Review Board. In addition, the study was cleared by three Alaska Native Health boards: the Alaska Native Tribal Health Consortium Health Research Review Committee, the Southcentral Foundation Research Oversight Committee, and the Yukon-Kuskokwim Health Corporation. All participants provided informed consent.

We follow the Practice Guidelines for Chronic Hepatitis B developed by the American Association for the Study of Liver Disease (AASLD) to make decisions regarding antiviral therapy and management. In general, all persons had baseline HBV DNA levels on the first specimen obtained after 2001 when HBV DNA testing became available in Alaska (Figure 1).(7) Those persons who were consented also had HBV genotype and sub-genotype testing performed. Persons who had a family history of HCC or had an HBV DNA level of over 2,000 IU/ml on first testing were followed with serial HBV DNA testing every 6 months. In addition, HBV DNA testing was repeated on all others who had elevated ALT or aspartate aminotransferase (AST) levels. Thus, only those with an HBV DNA level of < 2,000 IU/ml on initial testing who had persistently normal ALT/AS T levels did not have repeat HBV DNA testing performed. For the purpose of this study, we restricted the cohort to those persons who were HBeAg-negative on the first sera drawn after January 2001. The following criteria were established by our group in 2001 for recommending a liver biopsy based on AASLD Guidelines and as feasible in our population of Alaska Native persons: 1) persons with elevated ALT or AST level and HBV DNA > 20,000 IU/ml on any occasion; 2) persons with elevated ALT or AST > 30 who have HBV DNA between 2,000 and 20,000 IU/ml on two or more occasions, 3) persons over 40 years of age who previously had an HBV DNA between 2,000 and 20,000. We also recommend that persons with a personal history of previously treated HCC or family history of HCC with HBV DNA levels of > 2,000 IU/ml be considered for liver biopsy and evaluated for antiviral therapy.

Liver biopsies were scored using the modified histology activity index score (HAI) of Knodell (8) and the Ishak (9) fibrosis score. Patients with moderate or severe hepatitis (HAI

≥ 9) and moderate or severe fibrosis (Ishak ≥ 2) who met current AASLD criteria for antiviral treatment were compared for factors predicting degree of liver involvement.

For the purposes of this analysis we used a level of ALT of 20 U/L or higher for women and ≥30 U/L for men as the upper limit of normal. An HBV DNA level > 2,000 IU was considered suggestive of immune active hepatitis B. A participant met the study's criteria for immune active hepatitis B if they had an ALT above the defined upper limit of normal and an HBV DNA > 2,000 IU/ml, as per NIH criteria, at any time during the study period from October 1, 2001 to December 31, 2008.(10)

Laboratory Testing

Serologic testing for HBsAg, anti-HBs and antibody to hepatitis B core antigen (anti-HBc) was performed using enzyme-linked immunoassay (Abbott Laboratories, Irving, Texas) at the Alaska Native Medical Center clinical laboratory by commercial assay. Between 2001 and 2006, HBeAg determinations were performed using the One-Step Hepatitis B 'e' Antigen Test Strip, and the anti-HBe status of each specimen was determined using the Maxi: Test Anti-HBe Rapid Test (IND Diagnostic Inc., Delta, BC, Canada) as per the manufacturer. Both these tests were validated using sera previously tested with the Abbott Laboratories enzyme-linked immunoassay kits. After 2006 HBeAg and anti-HBe were performed by ELISA.

HBV viral load determination

Routine HBV viral load measurements conducted from 2001 and 2008 were determined using a Real-Time Quantitative polymerase chain reaction (PCR) assay with a lower limit of detection of approximately 10,000 copies/ml (200 IU/ml). Briefly, HBV DNA was extracted from stored serum specimens using the MagNA Pure Compact System and Total Nucleic Acid Extraction kit as per the manufacturer (Roche Diagnostics, Indianapolis, IN). Subsequently, a 118-bp fragment of the core gene was amplified and HBV viral loads were determined by comparison to a set of HBV DNA standards amplified in parallel with the samples being tested.(11) After 2008, HBV viral load measurements were also determined by Real-Time Quantitative PCR assay using the Roche Diagnostics COBAS® Ampliprep/ COBAS® Taqman system (Roche Diagnostics, Indianapolis, IN) with a lower limit of detection of approximately 100 copies/mL (20 IU/mL). DNA extraction, nested PCR, DNA sequencing and genotyping were performed as previously described. (12)

HBsAg Levels

HBsAg levels were determined on sera obtained at the time of liver biopsy using the ADVIA (Siemens Diagnostics) testing platform at Beth Israel Deaconess Medical Center at Harvard Medical School, Boston Massachusetts. HBsAg levels were analyzed by level of < 1,000 or ≥ 1,000 IU/ml and by mean geometric titer (GMT).

Statistical Analysis

Comparisons of proportions were made using chi-square, Fisher's exact, or randomization tests as appropriate. Logistic regression analysis was used for adjusted comparisons. Mean HAI and Ishak scores were compared using t-tests. Deaths were considered liver related if

they had the following ICD10 codes: K701-753, K758-769, B150/9, B159-162, B169-172, B178, B188-190, B942, C220-4, C227, or C229. All p-values are two-sided. P<0.05 was considered statistically significant.

Results

Prevalence of Immune Active Chronic Hepatitis B Infection

A total of 777 participants with chronic HBV infection were negative for HBeAg on their first serologic testing after October 1, 2001. The mean age of the cohort at the start of the follow-up was 38.6 years and 420 (54.1%) were male. No patients were documented to be positive for antibody to human immunodeficiency virus (HIV) and only two were positive for hepatitis C antibody and HCV RNA. During the study period, 201 (25.9%) persons met the criteria for immune active chronic hepatitis B and were included in this analysis. The characteristics of those who did and did not meet the criteria for immune active hepatitis B HBV are displayed in Table 1). HBV genotype was determined for 708 (91%) of the participants. Five HBV genotypes have previously been identified in this population, genotypes A1, B6, C, D, and F1.(12, 13) Compared to the other four genotypes, persons infected with genotype D had significantly lower prevalence of immune active chronic hepatitis B (p=0.007). Men were more likely than women to have immune active HBV infection (p=0.018). This difference remained after adjustment for HBV genotype (p=0.024) [Table 2]. The association of gender and active status was similar across genotypes; no significant differences among genotypes were observed (p=0.924).

No difference was found in the proportion of persons aged ≥40 years compared with those <40 years who had immune active hepatitis during the study period (25% vs. 27%; p=0.62). Interestingly, persons with a BMI ≥30 were significantly less likely than persons with a BMI <30 to have immune active hepatitis (23% vs. 30%; p=0.037). However, men were found to have higher BMI than women and after adjusting for gender, the relationship between higher BMI and lower prevalence of immune active HBV was no longer significant (p=0.09). Also no differences in the mortality rate among those with and without immune active disease were found during the study interval (p=0.810). Using a logistic model for immune active HBeAg-negative hepatitis, only male gender was associated with increased risk (p=0.023), and HBV genotype D was associated with a reduced risk (p=0.006) for immune active HBV. We also examined the relationship between BMI and HBV Genotype in Table 3. Adjusting for gender, the overall test for trend was not statistically significant for decreasing immune active HBeAg-negative hepatitis with increasing BMI (p=0.089).

We next examined the maximum ALT level that occurred during the seven years of prospective follow-up (Table 4). Males had significantly more abnormal ALT levels than females, both in terms of ALT above the normal limit defined (p=0.018) and by the proportion of ALT levels above 10 times the upper limit of normal (ULN) (p=0.040). However, no differences were found across HBV genotypes for maximum ALT level (P=0.76), levels greater than twice ULN (P=0.136) or maximum levels greater than 10 times ULN (P=0.538).

Liver Biopsy in Patients with Immune Active Chronic Hepatitis B Infection

Percutaneous liver biopsy was obtained in 46 (23%) of the 201 participants who met the study criteria for immune active hepatitis. Liver biopsy was performed significantly more often in patients with ALT greater than twice the ULN compared with those with ALT levels between one and two times the ULN (p=0.028) and more frequently in those who had one or more HBV DNA values that exceeded 20,000 IU/ml than in those with HBV DNA <20,000 IU/ml (p<0.001). There were no differences in the proportion of persons undergoing biopsy by age, gender, or BMI. However, a significantly higher proportion of persons infected with HBV genotypes C (47%) and F (35%) underwent liver biopsy than those with A1 (7%), B6 (17%), or D (18%) (p=0.005; 5 way comparison).

Factors predictive of moderate or severe liver inflammation (HAI≥9) or fibrosis (Ishak ≥2) among those who had liver biopsy are shown in Table 5 and Figure 1. We found no statistical differences in age, sex or HBV genotype. However, we did find significant differences among persons with ALT > vs. < twice ULN (p=0.001) and in those with HBV DNA levels > 20,000 vs. those between 2,000 and 20,000 IU/ml (p=0.001) [Table 5]. The proportion of persons with Ishak score ≥ 2 was significantly higher, 62% (16/26), for those with at least one HBV DNA level that exceeded 20,000 IU/ml compared to only 11% (2/19) for those whose levels were above 2,000 but never exceeded 20,000 IU/ml (p<0.001). Furthermore, of those in the immune active phase who had at least one ALT level > twice the ULN, 58% (18/31) had Ishak fibrosis score of ≥ 2 vs. 0% (0 of 14) whose ALT levels never exceeded twice the ULN (p=0.001). For persons with abnormal ALT levels less than twice the ULN, the mean HAI score was 1.7 vs. 5.7 for those with ALT levels ≥ twice the ULN (p<0.001); the mean Ishak scores were 0.14 vs. 2.0 for those, whose ALT was less than twice the ULN compared to those with one or more levels ≥ twice the ULN respectively (p<0.001). The mean HAI score for those whose HBV DNA was above 2,000 IU/mL but never reached 20,000 IU/ml was 3.1 and 5.5 for those who reached 20,000 IU/ml or higher during the study period (p=0.023) and the mean Ishak score was 0.53 vs. 2.1 respectively (p=0.001).

Thirteen of the 777 patients who began the study period as HBeAg-negative had one or more reversions to HBeAg-positive. Reversions were more frequently seen in persons infected with HBV genotype C compared to the other four genotypes (p=0.003), those with at least one HBV DNA level of >20,000 IU/ml (p=0.003) and those with at least one ALT > twice the ULN (p=0.023). No differences were seen in the frequency of reversions by age, sex, or BMI.

During the study period, 65 of the 777 persons died. Of those in the immune active phase, 16 (8.7%) of 201 died, two of whom had a liver-related death (both had HCC). Of the 576 persons who did not meet the criteria for immune active disease, 49 (8.5%) died, and six had a liver-related death. Two had HCC, one had bile duct carcinoma, and three had end stage liver disease; none had an HBV DNA level above 2,000 IU/ml.

HBsAg levels were performed from stored sera obtained at the time of or within 6 months before or after liver biopsy. When stratifying by HBsAg titer <1,000 IU/ml or ≥ 1,000 IU/ml, no significant differences were found in HAI or fibrosis scores. The HBsAg GMT

was not significantly different for those with HAI liver biopsy score of ≥9 vs. < 9 (205 vs. 525 IU/ml; p=0.449) or Ishak fibrosis scores of ≥2 vs. < 2 (249 vs. 726IU/ml; p=0.154).

Discussion

Our study examined a population-based cohort of persons who were HBeAg-negative and were followed prospectively for 8 years to determine the proportion who met the NIH criteria for immune active HBV; namely at least one elevated ALT level and one HBV DNA level of 2,000 IU/ml or greater.(10) This is the largest population-based prospective study of HBeAg-negative persons to date using serial HBV DNA and ALT levels that looked for immune active hepatitis. The cohort also included persons infected by five different HBV genotypes. We discovered several observations that greatly enhance our understanding of the natural history of HBeAg-negative HBV infection. We found that immune active HBV infection was significantly more common among men than among women and less likely among persons infected with HBV genotype D2 or D3 than those infected with genotypes, A2, B6, C2 or F1. While we found an inverse association with BMI and active liver disease; this association disappeared after adjusting for gender. Thus we were unable to show any association between BMI and severity of liver disease in our cohort. We also observed that patients with immune active hepatitis who had levels of aminotransferase levels above both twice and ten times the upper limit of normal were more likely to have been infected with HBV genotype C. HBV genotype C was found to be associated with a prolonged period of HBeAg positivity in this cohort from a previous study and a greater risk of developing cirrhosis or HCC than genotype B in prospective outcome studies conducted in Asia where this genotype is commonly found.(14-16). We also found that persons in the immune active phase with either ALT levels twice the ULN or HBV DNA levels above 20,000 IU/ml were more likely to have more advanced liver disease on biopsy.

The majority of persons with chronic HBV infection eventually become negative for HBeAg. However the proportion of those who have active liver disease after loss of HBeAg or who develop it over time is unknown, though has been estimated to be between 20% to 40%.(17) In addition, the proportion of HBeAg negative persons who have active liver disease varies by HBV genotype and thus will likely vary in different geographic locales, depending on the dominant HBV genotypes present.(15) Furthermore, among patients in the HBV immune active phase, the factors associated with the presence of moderate to severe disease on examination of hepatic pathology that fulfill the criteria for antiviral treatment is unknown. Again, this is because population-based studies of HBV with longitudinal clinical and laboratory data collection are few. This population-based cohort represents five of the eight known HBV genotypes found worldwide and, for those reasons, these findings are likely to apply to populations where one or more of these five genotypes is found, including most of central and southeast Asia, Europe, the Arctic, the Pacific Islands, North Africa, the United States and parts of South America.(15) In this study, we found that levels of both HBV DNA and ALT fluctuated widely in HBV infected individuals who were HBeAg-negative. Thus, relying on a single measurement of these levels in infected persons could be misleading. Cross sectional clinic-based studies have reported that approximately one quarter of persons with HBV DNA levels of between 2,000 and 20,000 IU/ml can have moderate or severe hepatitis on liver biopsy.(18-20) Some experts have recommended that

persons with an HBV DNA above 2,000 IU/mL and any elevation of ALT should be treated with antiviral therapy without the need for a liver biopsy.(21) This study identifies which patients in the immune active phase are more likely to have mild disease on liver biopsy and which have the highest probability of having moderate or severe liver inflammation or fibrosis. We found that those persons who had one or more elevation of HBV DNA of 2,000 IU/ml or greater but had no determinations above 20,000 IU/ml [the criteria for treatment under the current Practice Guidelines from the American Association for the Study of Liver Diseases (AASLD)]had only an 11% probability of having moderate or severe liver fibrosis on biopsy.(7) Thus treating persons with an elevated ALT and HBV DNA above 2,000 but below 20,000 IU/ml without a liver biopsy could result in the use of expensive antiviral agents in many persons for whom treatment is not currently recommended in evidenced-based guidelines established by National and International Liver Societies.(7, 22, 23) In contrast, those persons who had one HBV DNA level above 20,000 IU/ml during the 8-year follow-up period and/or one ALT level of above twice the upper limit of normal were more likely to have moderate to severe inflammation and/or moderate or severe fibrosis on liver biopsy and could benefit from antiviral therapy. A previous study supports our findings, for which no patients whose HBV DNA level remained between 2,000 and 20,000 IU/ml had more than mild liver inflammation or a fibrosis score of > 1 compared to most whose level exceeded 20,000 IU/ml. (24)

In those with immune active hepatitis, we found no relationship between the GMT of HBsAg levels at the time of biopsy in those with mild vs. moderate to severe liver disease. It would be helpful if HBsAg levels could predict the severity of liver disease and thus decrease the need for liver biopsy. A larger study should be conducted to confirm that our findings are accurate. In a study from Italy, HBsAg and HBV DNA levels were shown to be helpful in predicting whether persons in the inactive phase will remain in this phase over a 3-year follow-up period. (24)

The REVEAL study, a large population-based study looking at adverse clinical outcomes of HBV infection, has shown that persons had a significantly high risk of developing HCC if they had an HBV DNA level of > 10^4 copies (2,000 IU/ml) at enrollment and an HBV DNA level of >10^5 copies (20,000 IU/ml) at the end of the study 11 years later, but HBV DNA levels in between these time periods were not reported. Though our cohort was on average more than a decade younger (mid 30s) at the start of the 8-year follow-up, than were patients in the REVEAL cohort (mid 40s), our study findings would likely support those of the REVEAL study in that our participants whose HBV DNA levels reached 20,000 IU/ml had a greater risk of an adverse outcome or more severe liver disease on biopsy, which could subsequently lead to a greater risk of cirrhosis or HCC. We now exclusively prescribe tenofovir or entecavir for all therapy-naïve patients who need antiviral treatment and have switched those who develop antiviral resistance who were treated earlier with lamivudine or adefovir, to the more potent agents.

The three patients who died of endstage liver disease (ESLD) during the study period did not meet the HBV DNA criteria for immune active HBV. However, these three patients had a history of heavy alcohol use (>50g / day) which has been indicated as a significant factor in

ESLD. (22) However further longitudinal follow up is needed to determine the effects of antiviral therapy in this cohort.

Our study has some limitations. First, we performed liver biopsy in about one quarter of persons who fit the NIH criteria with immune active hepatitis. There are several reasons why more persons did not have liver biopsies. Primarily, most of our patients live in remote communities that are not connected to the road system and air transportation to Anchorage is required for a biopsy. Travel from these remote areas can be over 1000 miles, and is difficult and expensive. Thus, not all patients that we recommend for liver biopsy are able to travel and have this procedure performed. Eight persons who had ALT levels above twice the ULN and HBV DNA levels above 20,000 were empirically put on antiviral therapy without a liver biopsy since they met the criteria for treatment and were unable or unwilling to come to Anchorage for a liver biopsy. Two other patients were elderly (above age 80) and three patients lived in assisted living because of severe physical or mental disabilities. In these individuals the decision of whether to treat was made without a liver biopsy. A second limitation is that we did not systematically determine the cause of the elevated ALT levels in all patients. Thus, some persons with HBV DNA levels above 2,000 may have had non-hepatitis B related causes that contributed to their elevated ALT levels such as alcohol use or non-alcoholic fatty liver disease. Third, since this cohort represents a much younger mean age compared to other hepatitis B cohorts, conclusions about the prevalence of moderate to severe disease may not be directly comparable and could change as the cohort ages. Finally, this cohort consists entirely of Alaska Native persons, over 80% of whom are of Yupik Eskimo. It is possible that specific characteristics of this population may exist that would make our findings less translatable to other populations. However, thus far, studies have not carefully examined ethnic and racial differences in HBV disease outcome, despite numerous studies across the world in diverse populations.(17)

In conclusion, to our knowledge, this is the first large population-based prospective cohort study that examined the prevalence of immune active disease among HBeAg-negative persons. This study cohort represents five of the eight HBV genotypes found worldwide, three of which, HBV genotypes C, A2 and D, account for a substantial proportion of the global HBV infection burden. We found that approximately one quarter of persons with HBeAg-negative chronic HBV infection were in the immune active phase at some time during the 8-year follow-up period. Furthermore we found that approximately 90% of patients whose HBV DNA levels never reached 20,000 IU/ml had only mild disease on biopsy. Our study, along with the prospective cohort study on patients with genotype D (24), suggests that clinicians should consider a liver biopsy to stage the degree of pathologic severity rather than empirically starting treatment in HBV infected patients with elevated ALT levels and an HBV level of between 2,000 and 20,000 IU/ml. Since only a minority of these patients meet the AASLD histological criteria for treatment, exposure to possible lifelong antiviral therapy may not be justifiable, given the high cost and attendant risk for drug resistance.

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List of Abbreviations

HBV	Hepatitis B Virus
HCC	hepatocellular carcinoma
ALT	alanine aminotransferase
HBeAg	hepatitis B “e” antigen
anti-HBe	antibody to HBeAg
HBsAg	hepatitis B surface antigen
AASLD	American Association for the Study of Liver Disease
AST	aspartate aminotransferase
anti-HBc	antibody to hepatitis B core antigen
PCR	polymerase chain reaction
end	stage liver disease
GMT	mean geometric titer

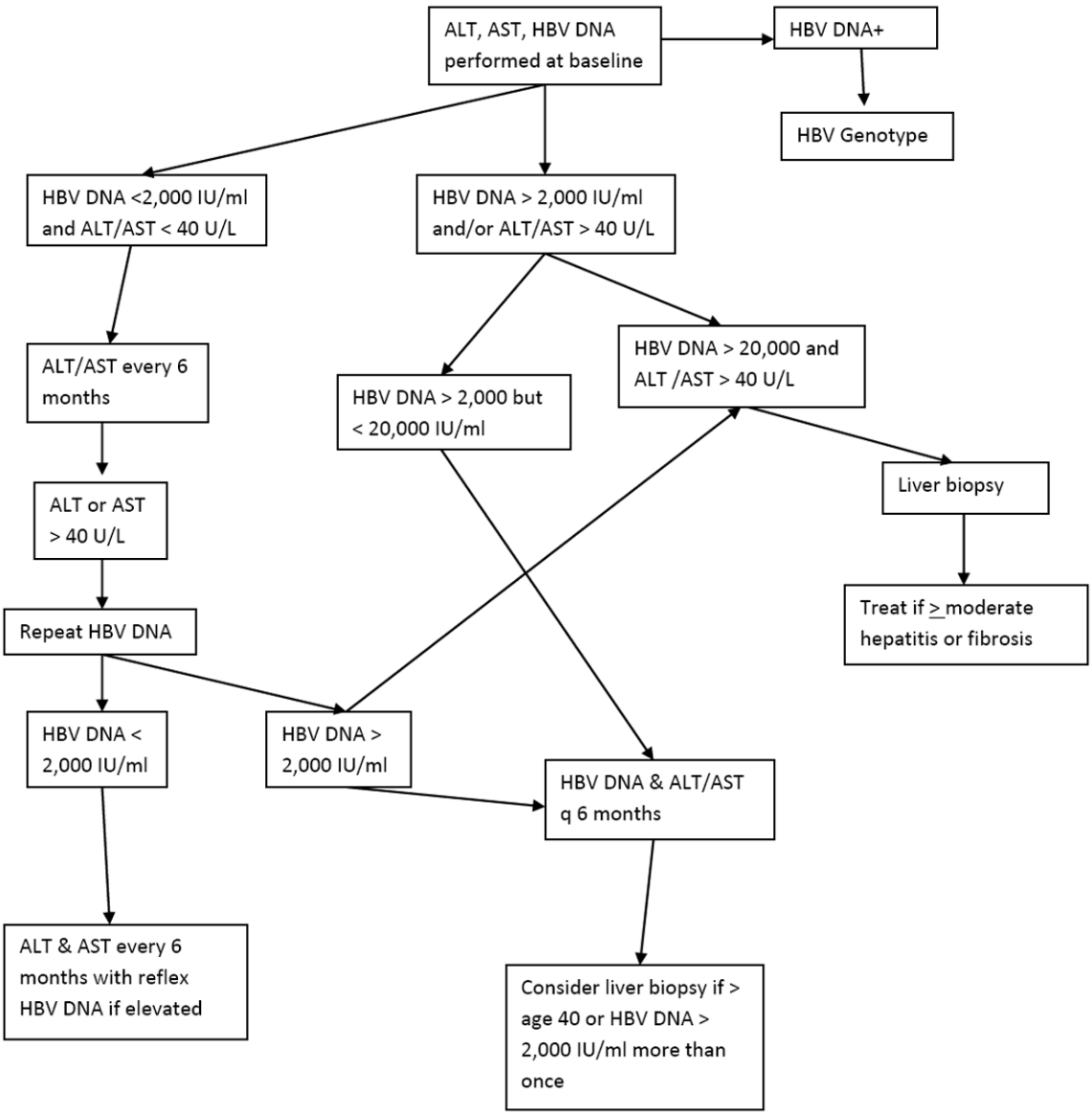

Figure 1.
Protocol for Following Persons with Chronic HBV Infection in Alaska

Table 1

Characteristics of Persons with Immune Active Chronic Hepatitis B[1] versus Inactive Chronic Hepatitis B in 777 Persons Persistently Negative for Hepatitis B "e" Antigen[2]:, Alaska, 2001 -2008

		Immune Active Number, (%)	Inactive Number.	
Total		201 (26%)	576	
Genotype	A	30 (28%)	76	5-way comparison; P=0.076
	B	7 (33%)	14	
	C	15 (35%)	28	
	D	93 (23%)	310	**D versus others; P=0.007**
	F	46 (34%)	89	A/B/C/F; P=0.775
	No result	10 (14%)	59	
Sex	Female	78 (22%)	279	**P=0.018**
	Male	123 (29%)	297	
Age	<40 years	124 (27%)	344	P=0.623
	≥40 years	77 (25%)	232	
	Mean (years)	37.7	38.9	P=0.293
BMI category[2]	<25	62 (30%)	142	3-way comparison; P=0.095
	≥25, <30	60 (30%)	139	<30 vs ≥30; **P=0.037**
	≥30	65 (23%)	220	Trend; p=0.051
Outcome	Died during interval	16 (25%)	49	P=0.810
	Alive during interval	185 (26%)	527	

[1] Immune active hepatitis B defined as ALT ≥ 20 in females and < 30 in males, plus HBV DNA of > 2,000 IU/ml

[2] BMI not obtained on 14 immune active and 75 inactive participants

Table 2

Number (%) with Immune Active HBeAg-negative Chronic Hepatitis by Gender and Genotype

		Females	Males	Comparing genders
Genotype	A	10/47 (21%)	20/59 (34%)	P=0.152
	B	0/10 (0%)	7/11 (64%)	**P=0.004**
	C	9/27 (33%)	6/16 (38%)	P=0.782
	D	36/181 (20%)	57/222 (26%)	P=0.170
	F	20/61 (33%)	26/74 (35%)	P=0.774
	No result	3/31 (10%)	7/38 (18%)	

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Table 3

Number of Persons (%) with Immune Active HBeAg-negative Chronic Hepatitis B by Gender and BMI

		Females	Males
BMI category	<25	23/92 (25%)	39/112 (35%)
	≥25, <30	24/87 (28%)	36/112 (32%)
	≥30	30/161 (19%)	35/124 (28%)
	Unknown	1/17 (6%)	13/72 (18%)
p-value for trend		0.183	0.276

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Table 4

Maximum ALT elevation among HBeAg-negative Persons with Chronic HBV Infection by genotype

		Not active hepatitis*	HBV-DNA ≥2000 MAX ALT			Total
			1-2x UNL	2-9x UNL	10x UNL	
Females			>20, ≤40	>40, ≤200	>200	
	A	37 (79%)	2 (4%)	8 (17%)	0	47
	B	10 (100%)	0	0	0	10
	C	18 (67%)	2 (7%)	7 (26%)	0	27
	D	145 (80%)	15 (8%)	19 (10%)	2 (1%)	181
	F	41 (67%)	11 (18%)	8 (15%)	1 (2%)	61
	Total**	279 (78%)	30 (8%)	45 (13%)	3 (1%)	357
Males			≥30, <60	≥60, <400	≥400	
	A	39 (66%)	14 (24%)	5 (10%)	1 (2%)	59
	B	4 (36%)	5 (45%)	2 (18%)	0	11
	C	10 (63%)	2 (12%)	3 (19%)	1 (6%)	16
	D	165 (74%)	29 (13%)	23 (10%)	5 (2%)	222
	F	48 (65%)	12 (16%)	10 (14%)	4 (5%)	74
	Total	297 (71%)	64 (15%)	46 (11%)	13 (3%)	420
Combined	A	76 (72%)	16 (15%)	13 (12%)	1 (1%)	106
	B	14 (67%)	5 (24%)	2 (10%)	0	21
	C	28 (65%)	4 (9%)	10 (23%)	1 (2%)	43
	D	310 (77%)	44 (11%)	42 (10%)	7 (2%)	403
	F	89 (66%)	23 (17%)	18 (13%)	5 (4%)	135
	Total	576 (74%)	94 (12%)	91 (12%)	16 (2%)	777

Table 5

Liver Biopsy Score in Persons Who Fulfilled the Criteria for Immune Active Disease

Biopsy Score		**HAI ≥9**		**Ishak ≥2**		**Either HAI ≥9 and/or Ishak ≥2**	
			p-value		**p-value**		**p-value**
Total		6/46 (13%)		18/45 (41%)		19/46 (41%)	
Genotype	A	0/2 (0%)		0/2 (0%)		0/2 (0%)	
	B	0/1 (0%)	1.0	0/1 (0%)	0.211	0/1 (0%)	0.276
	C	0/7 (0%)		5/7 (71%)		5/7 (71%)	
	D	2/17 (12%)		6/17 (35%)		6/17 (35%)	
	F	1/16 (6%)		4/15 (27%)		5/16 (31%)	
	No result	3/3 (100%)		3/3 (100%)		3/3 (100%)	
Sex	Female	2/19 (11%)	1.0	6/18 (33%)	0.543	6/19 (32%)	0.364
	Male	4/27 (15%)		12/27 (44%)		13/27 (48%)	
Age	<40 years	2/27 (7%)	0.213	8/26 (31%)	0.218	9/27 (33%)	0.233
	≥40 years	4/19 (21%)		10/19 (53%)		10/19 (53%)	
BMI category	<25	3/18 (17%)	0.299	9/18 (50%)	0.536	9/18 (50%)	0.474
	≥25, <30	0/14 (0%)		4/13 (31%)		4/14 (29%)	
	≥30	2/13 (15%)		4/13 (31%)		5/13 (38%)	
Outcome	Died during interval	2/4 (50%)	0.077	3/4 (75%)	0.286	3/4 (75%)	0.292
	Alive during interval	4/42 (10%)		15/41 (37%)		16/42 (38%)	
Max ALT levels							
	≤ 2 xULN	0/15 (0%)	0.157	0/14 (0%)	**<0.001**	0/15 (0%)	**<0.001**
	>2 xULN	6/31 (19%)		18/31 (58%)		19/31 (61%)	
	≤ 10 xULN	3/40 (8%)	**0.022**	13/39 (33%)	**0.031**	13/40 (33%)	**0.003**
	>10 xuln	3/6 (50%)		5/6 (83%)		6/6 (100%)	
Max HBV DNA							
	2,000-20,000	1/20 (5%)	0.212	2/19 (11%)	**0.001**	2/20 (10%)	**<0.001**
	>20,000	5/26 (19%)		16/26 (62%)		17/26 (65%)	